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Structural Features of Human Immunoglobulin G that Determine Isotype-specific Differences in Complement Activation

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Summary

Although very similar in sequence, the four subclasses of human immunoglobulin G (IgG) differ markedly in their ability to activate complement. Glu318-Lys320-Lys322 has been identified as a key binding motif for the first component of complement, C1q, and is present in all isotypes of Ig capable of activating complement. This motif, however, is present in all subclasses of human IgG, including those that show little (IgG2) or even no (IgG4) complement activity. Using point mutants of chimeric antibodies, we have identified specific residues responsible for the differing ability of the IgG subclasses to fix complement. In particular, we show that Ser at position 331 in $\gamma 4$ is critical for determining the inability of that isotype to bind C1q and activate complement. Additionally, we provide further evidence that levels of C1q binding do not necessarily correlate with levels of complement activity, and that C1q binding alone is not sufficient for complement activation.

The classical pathway of complement activation is initiated by immune complexes composed of antigen and either IgM or IgG Abs. Although the C region domains of all four human IgG subclasses share virtually identical amino acid sequences, they differ markedly in their ability to activate complement. Studies of mouse–human chimeric Abs have shown that IgG3 and IgG1 are effective in activating complement, IgG2 fixes complement poorly, and IgG4 appears completely deficient in the ability to activate complement (1, 2). The structural bases for these isotype-specific differences in complement activation have not been defined and are the subject of this investigation.

The hinge region, which separates the Fab from the Fc, has been implicated in determining these isotypic differences in part because the sequences of the hinge regions, in contrast to the other C region domains of the γ isotypes, are remarkably diverse (2, 3) and also because two hinge-deleted human IgG1 myeloma proteins lost both the ability to activate complement and to bind its first component, C1 (4). Additionally, hinge length and Fab arm segmental flexibility of IgG Abs from different species were found to correlate with the ability to activate complement (2, 5). Recently, however, we and others (6, 7) using genetically engineered Abs have shown that whereas a hinge region is essential for C1q binding and complement activation, the hinge does not appear to determine isotypic differences in these functions, and there is not an absolute correlation between segmental flexibility and the ability to activate complement. An IgG3 with the rigid hinge of IgG4 exhibits significant complement activation ability and an IgG4 with the hinge of IgG3, although as flexible as wild-type IgG3, displays no detectable complement activity. It thus appears that it is not the short, inflexible hinge but other structural features located in the H chain that lead to the inactivity of IgG4.

C_H2 has been implicated as the domain responsible for isotypic differences in complement fixation. Earlier Ab fragmentation studies (8–10) suggested a crucial role for C_H2 in activating complement by showing that Fab (IgG depleted of C_H3) and C_H2 fragments bind C1 and activate complement, whereas Fab and C_H3 fragments do not show any activity. Moreover, aglycosylated IgG (lacking carbohydrate side chains in C_H2) is unable to activate complement (11, 12). Domain-shuffled chimeric Abs provide further direct evidence that the isotypic variations in complement activity are determined by this domain. IgG2 with the C_H2 domain from IgG3 performs complement-mediated cell lysis almost as well as wild-type IgG3, and the replacement of C_H2 in IgG3 with the corresponding domain from IgG2 decreases its capacity to fix complement to a level comparable with that of wild-type IgG2 (13). By contrast, the exchange of C_H1 or C_H3 domains has no effect on complement activation (7, 13). Studies of domain switch Abs of murine IgG subclasses also confirmed the importance of C_H2 in determining the isotypic differences (14). We further localized the structural features leading to the inactivity of human IgG4 to the COOH-terminal part of C_H2, from residues 292 to 340 (13).

In the present study we identify specific polymorphic amino acids in the C_H2 domain of human IgG that account for sub-

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class differences in complement activation. Mutations introduced into chimeric IgG1, IgG3, or IgG4 show that the presence of Ser instead of Pro at residue 331 in wild-type IgG4 contributes significantly to its inability to bind C1q and activate complement. In addition, we provide evidence that substitutions at residues 276 and 291 can influence the ability to activate complement, although apparently not by affecting C1q binding, suggesting the involvement of additional Ab-dependent steps in the classical pathway of complement activation. Other polymorphic residues in C_H2 are also discussed.

Materials and Methods

In Vitro Mutagenesis and Construction of Chimeric IgG Molecules. The expressed Vκ and V_H genes from the mouse anti-dansyl (DNS)[1] hybridoma 27-44 were joined to human Cκ in the pSV2ΔH*neo* expression vector and to human IgG H chain in the pSV2ΔH*gpt* vector, respectively (15). The human genomic γ C region genes were cloned as SalI/BamHI cassettes. This makes it convenient to shuffle the genes between different plasmids and to join different H chain genes to the same V_H.

The SalI/BamHI cassettes containing human Cγ genes were cloned into the polylinker region of M13mp18 or M13mp19 for mutagenesis. To construct the intra-C_H2 domain switch variants of IgG1 and IgG4, the cloned IgG genes were digested with SalI and SacII (which cleaves within C_H2) and the SalI/SacII fragments were reciprocally exchanged between γ1 and γ4. Oligonucleotide-mediated site-directed mutagenesis was done based on the two-primer method of Zoller and Smith (16) with modifications and the mutations confirmed by sequencing.

Production of Transfectoma Proteins. P3X63Ag8.653, an Ig non-producing mouse myeloma cell line, was transfected simultaneously with the H and L chain expression vectors by electroporation (17). Transfectants were selected with G418 (Gibco, Grand Island, NY) at 1.0 mg/ml, and surviving clones were screened for Ab production by ELISA using DNS/BSA-coated plates. The amount of bound chimeric Ab was determined using alkaline phosphatase–conjugated polyclonal goat Ab (Sigma Chemical Co., St. Louis, MO) against human IgG C regions. Clones producing large quantities of anti-DNS Ab were expanded and maintained in IMDM containing 5% calf serum. Chimeric Abs were purified by DNS-coupled affinity chromatography as described previously (12). The concentrations of purified Abs were determined by a bicinchoninic acid–based protein assay (BCA; Pierce, Rockford, IL).

Complement-mediated Hemolysis. SRBC were coated with DNS-BSA (0.25 mg/ml DNS-BSA, 5% SRBC, in 150 mM NaCl, 0.25 mM $CrCl_3$, pH 7.0, for 1 h at 30°C) and loaded with ^{51}Cr-sodium chromate (Amersham Corp., Arlington Heights, IL). The free ^{51}Cr-sodium chromate was removed by washing the cells three times in 10 ml of fresh gel-HBSS buffer (0.01 M Hepes, 0.15 M NaCl, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 0.1% gelatin, pH 7.4). Chimeric Abs in gel-HBSS at various concentrations were added to round-bottomed, 96-well plates (Corning Glass Works, Corning, NY) in a volume of 50 μl. Then 50 μl of 2% ^{51}Cr-loaded SRBC and 10 times the amount of guinea pig complement (Colorado Serum Co., Denver, CO) required to produce lysis of 50% of the cells, preabsorbed against unlabeled SRBC, in a volume of 25 μl were added to each well sequentially. The plates were incubated at 37°C for 45 min, unlysed SRBC were pelleted by centrifugation of the plate, and 50 μl of supernatant counted in a gamma counter. Each point was assayed in triplicate and the percent lysis calculated. For Abs that contained a mutation that restored activity, multiple protein preparations were analyzed.

C1q Binding. All steps were carried out in HBSS plus 0.02% sodium azide and plates were washed six times between each step with the same buffer. 96-well plates (Immulon-2; Dynatech Laboratories Inc., Chantilly, VA) were coated with 100 μl/well DNS-BSA (40:1 substitution ratio, 10 μg/ml, overnight at 4°C) then blocked with 200 μl/well 3% BSA (overnight at 4°C). All Abs were diluted in 1% BSA, applied in a volume of 100 μl/well, and incubated at room temperature for 2 h or overnight at 4°C. All assays were carried out in quadruplicate. First, anti-DNS Abs were bound at a concentration of 20 μg/ml. The plates were washed and normal human serum diluted to 0.25% was applied in a volume of 100 μl at 37°C for 2 h. The detecting Ab was goat anti-human C1q (Atlantic Antibodies, Stillwater, MN) diluted 1:10,000, and the developing agent was swine anti-goat IgG–alkaline phosphatase conjugate (Boehringer Mannheim, Indianapolis, IN) diluted 1:20,000. 100 μl *p*-nitrophenyl phosphate (Sigma Chemical Co.) at 0.5 mg/ml in diethanolamine was added as phosphatase substrate, and the OD read at 410 nm.

Results

Our previous domain switch experiments clearly demonstrated that the C_H2 domain of IgG contains sites important for interacting with C1q and initiating activation of the complement cascade (13). Human IgG1, IgG3, and IgG4 exhibit very few polymorphisms in their C_H2 domains (Table 1), but differ profoundly in their ability to activate complement by the classical pathway. We set out to establish which amino acid polyporphisms determine the isotype-specific differences in complement activation. To address this question, a set of

Table 1. *Polymorphisms in C_H2 among IgG1, IgG3, and IgG4*

Residue	IgG1	IgG3	IgG4
234*	Leu‡	Leu	Phe
268	His	His	Gln
274	Lys	Gln	Gln
276	Asn	Lys	Asn
291	Pro	Leu	Pro
–	–		–
296	Tyr	Tyr	Phe
300	Tyr	Phe	Tyr
327	Ala	Ala	Gly
330	Ala	Ala	Ser
331	Pro	Pro	Ser

* Residue number is based on the EU numbering system.

‡ Sequences of IgG subclasses are taken from Kabat et al. (3) and confirmed by sequence analysis of the H chain genes used in these experiments.

(–) Position of C_H2 exchange in IgG (1/4) and IgG (4/1) hybrid proteins.

[1] *Abbreviations used in this paper:* DNS, dansyl.

Table 2. *Nomenclature and Constructs of C Region Domains for Chimeric Abs Used in this Study*

Name	Region Exons C_H1 H C_H2 C_H3	Description
IgG1		Wild type IgG1
IgG1(Lys276)		IgG1 Asn276→Lys
IgG1(Leu291)		IgG1 Pro291→Leu
IgG1(Ser330)		IgG1 Ala330→Ser
IgG1(Ser331)		IgG1 Pro331→Ser
IgG3		Wild type IgG3
IgG3(Asn276)		IgG3 Lys276→Asn
IgG3(Ser331)		IgG3 Pro331→Ser
IgG4		Wild type IgG4
IgG4(Tyr296)		IgG4 Phe296→Tyr
IgG4(Ala330)		IgG4 Ser330→Ala
IgG4(Pro331)		IgG4 Ser331→Pro
IgG(4/1)		4•4•4/1•1 hybrid
IgG(1/4)		1•1•1/4•4 hybrid
IgG 1/4 (Pro331)		IgG(1/4) Ser331→Pro

(⊓) Location of mutated amino acids.

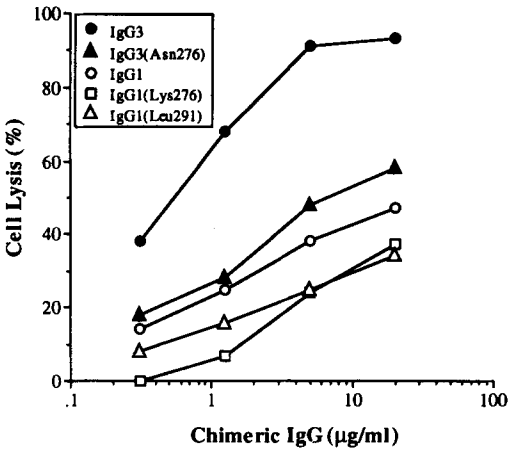

Figure 1. Complement-mediated hemolysis by wild-type and point mutants of IgG1 and IgG3. ^{51}Cr-loaded, DNS-BSA–coated SRBC were incubated with varying concentrations of Ab in the presence of guinea pig complement and percent cell lysis calculated. IgG3 mutated to resemble IgG1 at position 276 shows an activity reduced approximately to the level of wild-type IgG1. IgG3-like mutants of IgG1 however, do not show increased complement activity, rather they too are impaired relative to wild type.

mouse–human chimeric Abs having identical antigen-combining sites (anti-DNS) and L chains but with different H chain C region domains were generated using previously described techniques (15, 18). The point mutations were made in the context of either wild-type human γ1, γ3, γ4, or a C region in which γ1 and γ4 were shuffled at amino acid 292, as described previously (13). The names of the mutant proteins and the amino acids mutated in their C_H2 domains are shown in Table 2. All chimeric Abs were purified from culture supernatants using DNS isomer affinity chromatography (12) and used in the complement-mediated hemolysis and C1q binding assays.

Analysis of Residues Polymorphic between IgG1 and IgG3. In previous studies we have consistently found anti-DNS IgG3 to be about 10 times more effective than IgG1 in complement consumption and complement-mediated cell lysis (2, 12). Within the C_H2 domain, human IgG1 and IgG3 differ from each other at only four residues: 274 (Lys vs. Gln), 276 (Asn vs. Lys), 291 (Pro vs. Leu), and 300 (Tyr vs. Phe) (Table 1). We focused our attention on the polymorphisms at residues 276 and 291. Indeed, we find that substitution of Lys276 with Asn in IgG3 (IgG3[Asn276]) reduces its ability to activate complement to a level comparable with that seen in IgG1 (Fig. 1). However, the reciprocal mutation, Asn 276→Lys in IgG1 (IgG1[Lys276]), does not improve its ability to activate complement but instead results in a protein with impaired ability. Therefore, whereas the Lys 276→Asn substitution can make IgG3 functionally similar to IgG1 in its ability to activate complement, none of the single amino acid changes tested suffice to improve IgG1 to the level of IgG3.

There was a lack of correlation between the ability of the Abs to carry out complement-mediated hemolysis and their ability to bind C1q. However, it should be noted that whereas guinea pig complement was used in the direct lysis assay, human complement was used to measure C1q binding. IgG3 was consistently more effective than IgG1 in complement-mediated hemolysis (Fig. 1), but both isotypes were equivalent in their ability to bind C1q (Fig. 2). The result obtained in the current study differs slightly from our previously observation that IgG3 was seen to bind C1q better than IgG1 (12). However, a different version of the C1q binding assay was used in the earlier study. It is interesting that whereas exchanging residues at position 276 in IgG1 and IgG3 clearly affects both molecules negatively in overall complement activation, neither mutation significantly affects C1q binding (Fig. 2). IgG1(Leu291), also binds C1q as well as wild type, but is impaired in the direct lysis assay.

Role of Pro331. IgG4 is unable to activate complement or bind C1q, a deficiency that can be localized to the COOH terminus of C_H2 (13). These studies had shown that the hybrid protein IgG1/4, in which the C region switches from IgG1 to IgG4 at amino acid 292, is unable to activate complement whereas IgG4/1, the molecule with the reciprocal exchange, can, albeit not as well as IgG1. Within C_H2, the IgG1/4 protein differs from IgG1 at four residues: Phe296 instead of Tyr, Gly327 instead of Ala, Ser330 instead of Ala, and Ser331 instead of Pro. The Ser/Pro polymorphism at residue 331 seemed to be a good candidate for contributing to the differences in activity, both because of the nature of the substitution and because of the position of Pro exposed on the surface of the protein (Fig. 3). Indeed, we find that substitution of Pro at position 331 in IgG4 (IgG4[Pro331]) results in a molecule that can now bind C1q and mediate complement-directed lysis (Figs. 2 and 4 *A*). When this substitution is made in the context of the IgG1/4 protein, the resulting mol-

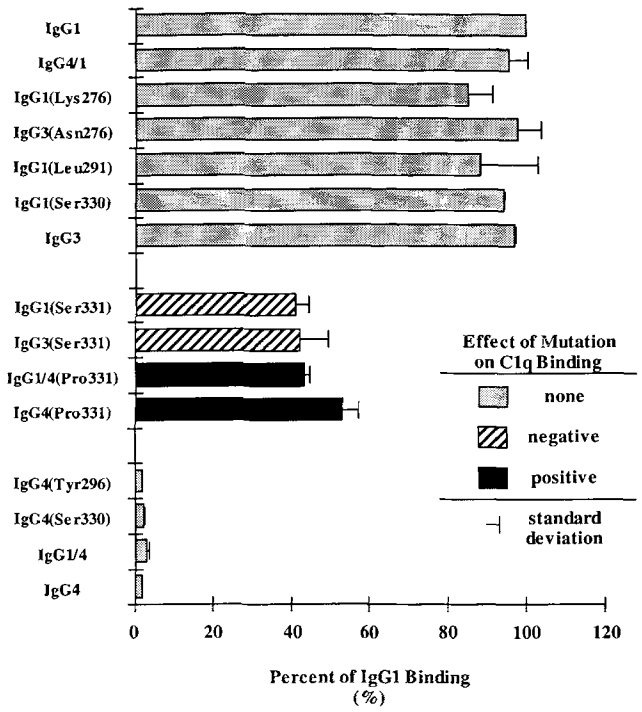

Figure 2. C1q binding by wild-type and point mutants of IgG1, IgG3, IgG4, and the 1/4 hybrid proteins. C1q bound to Ag–Ab complexes on a microtiter dish was detected with goat anti-C1q Ab and swine anti-goat IgG conjugated to alkaline phosphatase. Substitution of Pro for Ser at position 331 in IgG4 and the IgG1/4 hybrid results in Abs now capable of binding C1q (■). The reciprocal mutation in IgG1 and IgG3 greatly reduces their ability to bind C1q although only to the level of the Pro331 mutants of IgG4 and IgG1/4 (▨). Our mutations at positions 276, 291, 296, and 330 in IgG1, IgG3, and IgG4 showed little or no affect on C1q binding (▩). Each protein was tested in quadruplicate and in two or more independent assays. Error bars represent the variation between assays.

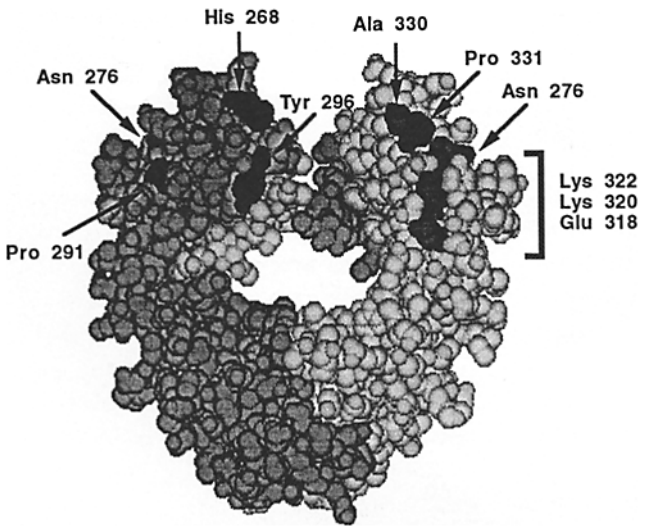

Figure 3. Three-dimensional structure of human IgG1 Fc. Amino acids that are discussed in the text are darkened and indicated by arrows. Ala327 is buried within the molecule. For clarity, one H chain is shaded slightly more than the other and associated carbohydrates are shaded like the opposing H chain. The graphic is based on the coordinates from Diesenhofer (23) and was produced using the program MacImdad (Molecular Applications Group, Stanford University, Stanford, CA).

ecule, IgG1/4(Pro331), also binds C1q and is even more efficient in complement-mediated lysis than IgG4(Pro331). To further prove that Pro331 is critical for complement activation, we created Ser331 mutants of both IgG1 and IgG3. The substitution of Ser for Pro at position 331 in IgG1 (IgG1[Ser331]) results in complete loss of the ability to carry out complement-mediated hemolysis (Fig. 4 *B*). It is interesting, however, that the molecule binds C1q as well as IgG1/4(Pro 331) (Fig. 2), which is quite effective in carrying out complement-mediated hemolysis (Fig. 4). The same substitution in IgG3 (IgG3[Ser331]) also profoundly impairs, but does not abolish, its ability to carry out complement-mediated hemolysis, and reduces its C1q binding to the intermediate level characteristic of IgG1/4(Pro331), IgG4(Pro331), and IgG1(Ser331).

Other Polymorphic Residues in IgG1 and IgG4. Although the replacement of Ser 331 with Pro in IgG4 partially restores its activity, IgG4(Pro331) is still far less efficient than wild-type IgG1 in activating complement. Thus, additional mutant proteins were made to investigate the role of other residues polymorphic between the two isotypes. Substitution of Ser for Ala are residue 330 in IgG1 (IgG1[Ser330]) did not significantly alter the complement activating or C1q binding ability of the molecule (Figs. 2 and 5). When the reciprocal substitution was made in IgG4 (IgG4[Ala330]) the Ab remained unable to activate complement or bind C1q. Similarly, substitution of the polymorphic residue at position 296 (IgG4[Tyr296]) left the molecule deficient in its ability to activate complement. Thus, we find no evidence that residues 296 or 330 determine isotype-specific differences in complement activation.

Discussion

Activation of complement through the classical pathway is initiated by the interaction of C1q and IgG or IgM in an immune complex. Previous studies (8–10) have indicated that the C_H2 domain of the H chain of IgG contains structures critical for complement activation and that complement-activating capacities can be transferred between active and inactive IgG subclasses with C_H2, whereas the exchange of C_H1, hinge, or C_H3 produces little or no effect (7, 13, 14). Three possible C1 binding sites have been proposed: His285-Arg292 (19), the residues on the last two antiparallel β strands (20), and Lys290-Glu295 (21). Based on protein engineering studies of mouse IgG2b, three charged amino acids (Glu318, Lys320, and Lys322) located on one β strand of C_H2 were proposed as constituting the essential C1q binding motif (22). However, this core binding motif is conserved in all four human IgG subclasses that exhibit dramatic differences in complement activation, including IgG4, which is completely inactive and does not bind C1q. Clearly, additional structural elements must determine the isotype-specific differences in IgG–complement interaction. We have now focused our attention on the polymorphic amino acids located in C_H2 which may determine these differences.

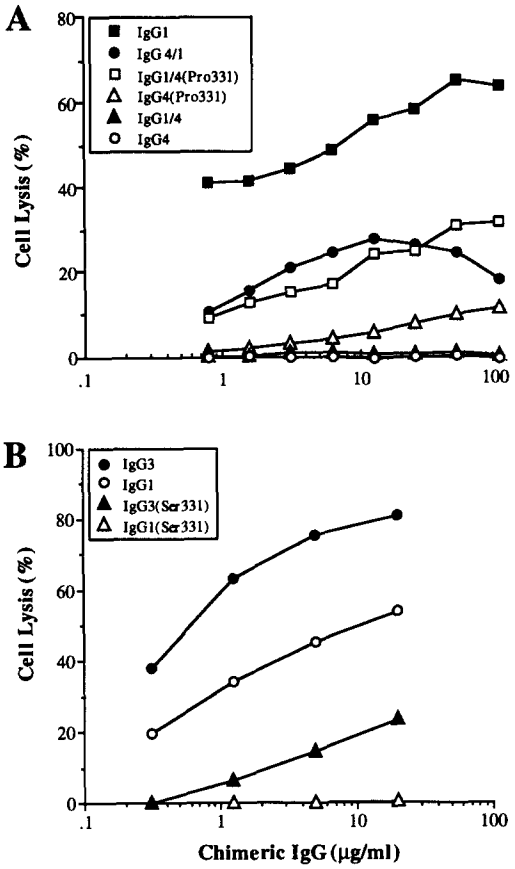

Figure 4. Complement-mediated hemolysis by IgG wild-type and position 331 mutants. (*A*) IgG4 and the IgG1/4 hybrid show no activity. However, substitution of Pro for Ser at position 331 results in Abs now capable of directing lysis. (*B*) Substitution of Ser for Pro at position 331 severely impairs the ability of IgG3 to direct lysis and completely abolishes IgG1 activity.

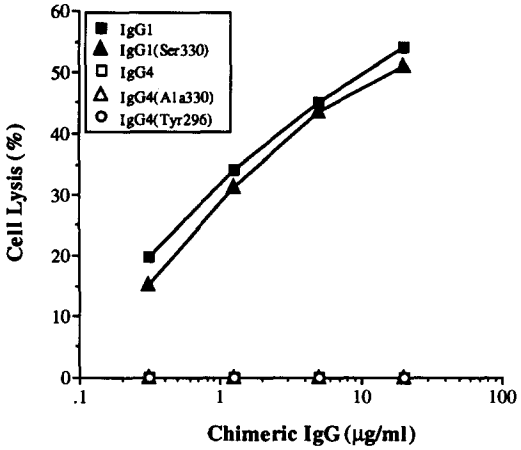

Figure 5. Complement-mediated hemolysis by wild-type and point mutants of IgG1 and IgG4. Mutating position 330 of IgG1 to resemble IgG4 does not affect the ability of the molecule to direct lysis. Similarly, IgG1-like position 296 and 330 mutants of IgG4 remain completely inactive.

IgG3, which is more efficient than IgG1 in complement consumption and complement-mediated cell lysis (2, 12), differs in C_H2 from IgG1 at only four amino acids (Table 1). In the three-dimensional structure (23), residue 276 is in close contact with the proposed 318-320-322 C1q binding motif (Fig. 3), and it is conceivable that the charged side chain of Lys276 in IgG3 contributes to the C1q–IgG interaction. In fact, we found that substitution of Lys276 with Asn in IgG3 (IgG3[Asn276]) reduces its complement-activating capacity to the level seen in IgG1. However, the reciprocal change in IgG1 (IgG1[Lys276]) does not increase its activity. Instead, the mutant Ab is somewhat impaired in its capacity to mediate cell lysis. Furthermore, although both mutations affect complement-mediated cell lysis, neither has a significant effect on C1q binding. These results suggest that the side chain of Lys276 is involved in some subsequent Ab-dependent step of the cascade and that in the context of IgG1, it does not assume a proper configuration for effective complement activation. Similarly, mutation at residue 291 in IgG1 from Pro to Leu (as found in IgG3) decreases its ability to activate complement without affecting C1q binding, again illustrating the importance of the context of the entire domain in determining the contribution of specific residues.

Human IgG4 is unable to bind C1q and is incapable of complement activation. It has been postulated that either reduced segmental flexibility (2, 5) or steric hindrance by Fab arms (24) caused by a short, rigid IgG4 hinge was responsible for the inactivity. However, recent Ab engineering experiments by us and others (6, 8) have provided evidence against this hypothesis. An IgG4 molecule with the flexible hinge region of IgG3 fails to bind C1q or activate complement, indicating that a structural lesion other than the rigid hinge leads to the inactivity of IgG4. By intra-domain exchange between IgG1 and IgG4 we have located the structures responsible for the inactivity of IgG4 to the COOH-terminal part of C_H2 (from residue 292-340) (13). We now show that residue 331 is a critical amino acid for determining the isotypic differences in complement activation. Substitution of Ser331 with Pro in IgG4 or the IgG1/4 hybrid results in Abs now capable of binding C1q and of complement-mediated lysis. In addition, the reciprocal exchange of Pro331 to Ser in IgG3 or IgG1 dramatically decreases or completely abolishes the capacity to activate complement. Residue 331 lies in a peptide loop between the last two β strands of C_H2 and is in close proximity to the 318-320-322 C1q binding motif. It is feasible that Pro331 is either part of the C1q binding site or is required for maintaining the appropriate structures for C1q–IgG interaction. Moreover, Pro436 in $C\mu3$ is analogous to Pro331 in $C\gamma2$ and a mutant IgM Ab with Pro436→Ser showed a >50-fold reduction in complement activity (25). This observation further supports a critical role for Pro331. It is interesting that the Pro331→Ser mutation in IgG3 also caused a 10-fold reduction in its affinity for the human high affinity Fcγ receptor (26). Therefore, the presence of Pro at residue 331 is important for both complement activation and Fc receptor binding.

The IgG1 (Ser331) mutant is of particular interest because, although it binds C1q, it fails to carry out lysis, demonstrating

clearly that C1q binding alone is not sufficient for complement activation. This conclusion is supported by previous results in which a mutant of IgG3 lacking carbohydrate in C_H2 could bind C1q but not consume complement (12). Our results suggest that amino acids other than Pro331 and polymorphic between IgG1 and IgG3 are important for Ab-dependent steps of the complement cascade besides C1q binding. A good candidate in this case is residue 274 since both $\gamma3$ and $\gamma4$ have Gln (neutral) in this position and $\gamma1$ has Lys (positive). Additional Ab-dependent steps that have been proposed and at which specific differences between IgG1 and IgG3 have been found are the deposition of C3 and C4 (27).

It is noteworthy that Ser331→Pro substitution in IgG4 or in IgG1/4 does not result in Abs as effective as IgG1 in complement activation or C1q binding. The hierarchy of activity is IgG1 > IgG1/4(Pro331) > IgG4(Pro331). Clearly, additional structural features of these molecules contribute to their different capacities. As shown in Table 1, IgG1 and IgG1/4(Pro331) are identical except for C_H3 and three polymorphic residues within C_H2: Tyr296Phe, Ala327Gly, and Ala330Ser. Residue 327 is mostly buried inside the molecule and is probably not directly involved in C1q binding. Residues 296 and 330 are exposed on the outer surface of the C_H2 domain (Fig. 3) and thus could be directly involved in complement–IgG interaction. Single amino acid substitutions at residue 296 or 330 in IgG4 however, did not have any effect on complement activation or C1q binding. Also, substitution of a Ser at position 330 in IgG1 did not decrease its ability to activate complement or lower its level of C1q binding. This result implies that the different activities of IgG1 and IgG1/4(Pro331) may be due to the combinatorial effect of these polymorphic residues. Introduction of multiple mutations at these residues should clarify this issue.

The fact that IgG1/4(Pro331) is more active than IgG4-(Pro331) in the direct lysis assay indicates that the NH_2-terminal part of the H chain also plays a role in determining isotype-specific differences in complement activation. The hinge region may contribute to this difference. In addition, three residues polymorphic between IgG1 and IgG4, Leu234 Phe, His268 Gln, and Lys274 Gln, are present in this portion of C_H2. Residue 234 is part of the lower hinge region and has been shown to be critical for Fc receptor binding (26, 28). However, we found that IgG4(Leu234) is unable to trigger complement activation and the reciprocal change, IgG3 (Phe234), is almost as active as wild-type IgG3 (29). The involvement of residue 274 in complement activation was thought to be unlikely since a mutation at this residue in murine IgG2b did not alter its activity (22). Moreover, the same amino acid, Gln, is found in both complement-active IgG3 and complement-inactive IgG4. In light of our results, however, this residue does warrant investigation. Residue 268 also seems to be a good candidate. As shown in Fig. 3, its side chain is exposed on the outer surface of C_H2 and a positively charged His present at this residue in IgG1 and IgG3 is substituted by a neutral Gln in IgG4.

The present study has demonstrated that multiple amino acids within the C_H2 domain determine the relative potency of the different human IgG isotypes in complement activation. We have shown that a single amino acid substitution (Ser331→Pro) in complement-deficient IgG4 is sufficient to impart some ability to bind C1q and activate complement and that the reciprocal mutations in IgG1 and IgG3 decrease their ability to bind C1q and to direct complement-mediated lysis. We have also produced several mutant Abs in which there is not a direct correlation between the ability to bind C1q and the ability to effect complement-mediated hemolysis. IgG1(Ser331), in particular, binds C1q but is incapable of complement-mediated hemolysis. These mutants provide compelling evidence for the existence of additional Ab-dependent steps in the classical pathway of complement activation. In addition, we have found that whereas it is relatively straightforward to produce Abs with reduced or loss of ability to activate complement, it much more of a challenge to produce genetically engineered Abs with improved ability to activate complement. Amino acid substitutions at one location can cause distortion of local, sometimes even distal three-dimensional structure. Thus, whereas loss of function of mutants may support the importance of specific residues as direct contacts for interacting molecules, they do not exclude the possibility that the residues may function instead by influencing the conformation of the binding site located elsewhere in the molecule. The importance of residue 331 in IgG–complement interaction has been demonstrated here by the inactivity of IgG1(Ser331) and IgG4/1(Ser331), but most convincingly by the acquisition of activity by IgG4 (Pro331) and IgG1/4(Pro331). Results obtained from this type of functional analysis of Ab molecules will facilitate the design of Igs that exhibit optimal combinations of effector functions allowing for more specific and defined manipulation of the immune system.

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